FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
0769.00140	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/582524	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/03548	October 27, 1999	October 17, 1998

TITLE OF INVENTION
ASSAYS FOR AUTOANTIBODIES

APPLICANT(S) FOR DO/EO/US
Peter John Burne; Bernard Rees Smith

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Date of Deposit 6-27-00

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Constance McLean
(Signature of person mailing paper or fee)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/582524	PCT/GB99/03548	0769.00140

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	69 - 20 =	49	x $18.00	**$882.00**	
Independent claims	2 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,722.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☒	**$861.00**	
SUBTOTAL =				**$861.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$861.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☒	**$40.00**	
TOTAL FEES ENCLOSED =				**$901.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$901.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **11-1449** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Kenneth I. Kohn
KOHN & ASSOCIATES
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SIGNATURE

Kenneth I. Kohn
NAME

30,955
REGISTRATION NUMBER

June 27, 2000
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

PETER JOHN BURNE ET AL.

Serial No.: Unknown

Filed: Herewith

For: ASSAYS FOR AUTOANTIBODIES

Our File No.: 0769.00140

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified patent application prior to examination on the merits as follows:

IN THE SPECIFICATION:

Page 1, after the title, please insert the following paragraph:

-- **CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a National Phase application concerning a filing under 35 U.S.C. 371, of International Patent Application Serial No. PCT/GB99/03548, filed October 27, 1999, which claims priority on British Patent Application Serial No. 9823397.6, filed October 27, 1998, both of which are incorporated herein by reference. –

IN THE CLAIMS:

Please cancel claims 1-81 and insert the following new claims therefor:

82. A method of screening a sample of body fluid for at least one autoantibody to at least one antigen, which method comprises:

(a) providing a source of said at least one antigen to said autoantibody;

(b) providing a substrate having immobilized thereto at least one antibody to said antigen of step (a);

(c) contacting said antigen source of step (a) with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) allowing said mixture obtained in step (c) to flow relative to said substrate of step (b) so as to allow said mixture to contact said antibody immobilized to said substrate;

(e) providing labelling means so as to permit monitoring of binding of said autoantibody and said antigen present in said mixture obtained in step (c); and

(f) monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

83. A method according to claim 82, wherein said antigen comprises a thyroid protein.

84. A method according to claim 83, wherein said thyroid protein includes thyroid stimulating hormone receptor.

85. A method according to claim 83, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase and thyroglobulin.

86. A method according to claim 82, which further comprises screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

87. A method according to claim 82, which comprises contacting in step (c) said antigen source and said sample of body fluid with at least one substantially non-immobilized antibody to said antigen.

88. A method according to claim 87, wherein said non-immobilized antibody is provided in substantially purified form.

89. A method according to claim 87, wherein said non-immobilized antibody is selected from the group consisting of a monoclonal antibody and an autoantibody to said antigen.

90. A method according to claim 82, wherein said monitoring in step (f) comprises observing a colorimetric change dependent on said binding of said autoantibody and said antigen present in said mixture of step (c).

91. A method according to claim 90, wherein said labelling means include colloidal gold.

92. A method according to claim 82, which further comprises providing a positive control that is present in the presence or absence of the autoantibody or autoantibodies being screened.

93. A method according to claim 82, wherein said mixture obtained in step (c) is allowed to flow along said substrate and interact with said antibody immobilized to said substrate.

94. A method according to claim 93, wherein at least said sample of body fluid is contacted with an application zone of said substrate, which

application zone is provided upstream on said substrate relative to said immobilized antibody, and wherein said mixture is allowed to flow from said application zone along said substrate so as to interact with said immobilized antibody.

95. A method according to claim 94, wherein said application zone includes said source of said antigen of step (a), and said mixture in step (c) is obtained by contacting said sample of body fluid with said antigen of said application zone.

96. A method according to claim 94, wherein said application zone further includes at least one substantially non-immobilized antibody to said antigen, and said mixture in step (c) is obtained by contacting said sample of body fluid and said antigen with said non-immobilized antibody present in said application zone.

97. A method according to claim 94, wherein said antigen source of step (a) and said sample of body fluid are contacted substantially remote from said substrate so as to provide said mixture of step (c), and said mixture is subsequently contacted with said application zone.

98. A method according to claim 97, wherein at least one reagent selected from said antigen source of step (a), said sample of body fluid and at least one substantially non-immobilized antibody to said antigen, is contacted substantially remote from said substrate so as to provide said mixture of step (c), and said mixture is subsequently contacted with said application zone.

99. A method according to claim 82, wherein said substrate is a membrane selected from the group consisting of nitrocellulose, cellulose acetate and polyamide.

100. A method according to claim 82, wherein said immobilized antibody is in substantially purified form.

101. A method according to claim 82, wherein said immobilized antibody is selected from the group consisting of an autoantibody to said antigen and a monoclonal antibody.

102. A method according to claim 82, wherein said sample of body fluid is selected from the group consisting of blood, plasma, serum and urine.

103. A method according to claim 82, which comprises screening said sample of body fluid for one said autoantibody.

104. A method according to claim 103, wherein said antigen includes a binding site to which either said autoantibody or said immobilized antibody can bind, whereby in step (d) binding of said immobilized antibody to said binding site is substantially precluded where said autoantibody has substantially bound to said binding site in step (c).

105. A method according to claim 82, which comprises screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein at least first and second antibodies to said antigen are immobilized on said substrate in step (b).

106. A method according to claim 105, wherein said antigen includes:

a first binding site to which either said first autoantibody or said first immobilized antibody can bind, whereby in step (d) binding of said first immobilized antibody to said first binding site is substantially precluded where said first autoantibody has substantially bound to said first binding site in step (c); and

a second binding site to which either said second autoantibody or said second immobilized antibody can bind, whereby in step (d) binding of said second immobilized antibody to said second binding site is substantially precluded where said second autoantibody has substantially bound to said second binding site in step (c);

wherein said first and second binding sites are substantially distinct sites on said antigen.

107. A method according to claim 82, wherein said antigen is provided with said labelling means.

108. A method according to claim 92, wherein said positive control comprises attaching to the substrate at least one control antibody to the antigen, which control antibody binds to a site on the antigen distinct to a binding site thereof for the autoantibody or autoantibodies being screened.

109. A method according to claim 87, wherein said non-immobilized antibody is provided with said labelling means, which non-immobilized antibody is capable of binding to a site on said antigen substantially distinct from a binding site for either (i) said autoantibody or autoantibodies being screened or (ii) said immobilized antibody, whereby in step (d), antigen is allowed to be substantially bound both to said immobilized antibody and to said non-immobilized antibody.

110. A method according to claim 87, which comprises screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein said non-immobilized antibody is capable of binding to a site on said antigen to which either said first or second autoantibody can bind and which is substantially distinct to a binding site on said antigen for said immobilized antibody, whereby in step (d) antigen is allowed to be substantially bound both to said immobilized antibody and to said non-immobilized antibody.

111. A method according to claim 110, wherein said antigen includes:

a first binding site to which either said first autoantibody or said immobilized antibody can bind, whereby in step (d) binding of immobilized antibody to said first binding site is substantially precluded where said first autoantibody has substantially bound to said first binding site in step (c); and

a second binding site to which either said second autoantibody or said non-immobilized antibody can bind;

wherein said first and second binding sites are substantially distinct sites on said antigen.

112. A method according to claim 110, wherein said non-immobilized antibody is provided with said labelling means.

113. A method according to claim 110, wherein said immobilized antibody comprises a first autoantibody to said antigen and said non-immobilized antibody comprises a second autoantibody to said antigen.

114. A method according to claim 87 which further comprises a positive control that is present in the presence or absence of the autoantibody or autoantibodies being screened, wherein the positive control comprises attaching to the substrate at least one control agent that can bind to the at least one substantially non-immobilized antibody.

115. A kit for use in screening a sample of body fluid for at least one autoantibody to at least one antigen, which kit comprises:

(a) a source of said at least one antigen to said autoantibody;

(b) a substrate having immobilized thereto at least one antibody to said antigen;

(c) means for contacting said antigen source with said sample of body fluid, so as to obtain a mixture wherein said antigen is

allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) means for allowing said mixture to flow relative to said substrate so as to allow said mixture to contact said antibody immobilized to said substrate;

(e) labelling means to permit monitoring of binding of said autoantibody and said antigen present in said mixture; and

(f) means for monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

116. A kit according to claim 115, wherein said antigen comprises a thyroid protein.

117. A kit according to claim 116, wherein said thyroid protein includes thyroid stimulating hormone receptor.

118. A kit according to claim 116, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase and thyroglobulin.

119. A kit according to claim 115, which further comprises means for screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

120. A kit according to claim 115, which further comprises a source of at least one substantially non-immobilized antibody to said antigen and means whereby said non-immobilized antibody can be contacted with said antigen source and said sample of body fluid.

121. A kit according to claim 120, wherein said non-immobilized antibody is provided in substantially purified form.

122. A kit according to claim 120, wherein said non-immobilized antibody is selected from the group consisting of a monoclonal antibody and an autoantibody to said antigen.

123. A kit according to claim 115, wherein said monitoring means comprise means for observing a colorimetric change dependent on said binding of said autoantibody and said antigen present in said mixture.

124. A kit according to claim 123, wherein said labelling means include colloidal gold.

125. A kit according to claim 115, which further comprises a positive control that is present in the presence or absence of the autoantibody being screened.

126. A kit according to claim 115, wherein said substrate comprises an application zone for at least said sample of body fluid, which application zone is provided upstream on said substrate relative to said immobilized antibody, whereby said mixture is allowed to flow from said application zone along said substrate so as to interact with said immobilized antibody.

127. A kit according to claim 126, wherein said application zone includes said source of said antigen, and said mixture is obtained by contacting said sample of body fluid with said antigen of said application zone.

128. A kit according to claim 126, wherein said application zone further includes at least one substantially non-immobilized antibody to said antigen, and means whereby said mixture is obtained by contacting said sample of body fluid and said antigen with said non-immobilized antibody present in said application zone.

129. A kit according to claim 126, wherein means are provided whereby said antigen source and said sample of body fluid are contacted substantially remote from said substrate so as to provide said mixture and means whereby said mixture is subsequently contacted with said application zone.

130. A kit according to claim 129, wherein means are provided whereby at least one reagent selected from said antigen source, said sample of body fluid and at least one substantially non-immobilized antibody to said antigen, is contacted substantially remote from said substrate so as to provide said mixture, and means whereby said mixture is subsequently contacted with said application zone.

131. A kit according to claim 115, wherein said substrate is a membrane selected from the group consisting of nitrocellulose, cellulose acetate and polyamide.

132. A kit according to claim 115, wherein said immobilized antibody is provided in substantially purified form.

133. A kit according to claim 115, wherein said immobilized antibody is selected from the group consisting of an autoantibody to said antigen and a monoclonal antibody.

134. A kit according to claim 115, wherein said sample of body fluid is selected from the group consisting of blood, plasma, serum and urine.

135. A kit according to claim 115, for screening said sample of body fluid for one said autoantibody, wherein said antigen includes a binding site to which either said autoantibody or said immobilized antibody can bind, whereby binding of said immobilized antibody to said binding site is

substantially precluded where said autoantibody has previously substantially bound to said binding site.

136. A kit according to claim 115, for screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein at least first and second antibodies to said antigen are immobilized on said substrate.

137. A kit according to claim 136, wherein said antigen includes:

a first binding site to which either said first autoantibody or said first immobilized antibody can bind, whereby binding of said first immobilized antibody to said first binding site is substantially precluded where said first autoantibody has previously substantially bound to said first binding site; an

a second binding site to which either said second autoantibody or said second immobilized antibody can bind, whereby binding of said second immobilized antibody to said second binding site is substantially precluded where said second autoantibody has previously substantially bound to said second binding site;

wherein said first and second binding sites are substantially distinct sites on the antigen.

138. A kit according to claim 115, wherein said antigen is provided with said labelling means.

139. A kit according to claim 125, wherein the positive control comprises attaching to the substrate at least one control antibody to the antigen, which control antibody binds to a site on the antigen distinct to a binding site thereof for the autoantibody or autoantibodies being screened.

140. A kit according to claim 120, wherein said non-immobilized antibody is provided with said labelling means, which non-immobilized antibody is capable of binding to a site on said antigen substantially distinct

from a binding site for either (i) said autoantibody or autoantibodies being screened or (ii) said immobilized antibody, whereby antigen is allowed to be substantially bound both to said immobilized antibody and to said non-immobilized antibody.

141. A kit according to claim 120 for screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein said non-immobilized antibody is capable of binding to a site on said antigen to which either said first or second autoantibody can bind and which is substantially distinct to a binding site on said antigen for said immobilized antibody, whereby antigen can be substantially bound both to said immobilized antibody and to said non-immobilized antibody.

142. A kit according to claim 141, wherein said antigen includes:

a first binding site to which either said first autoantibody or said immobilized antibody can bind, whereby binding of immobilized antibody to said first binding site is substantially precluded where said first autoantibody has previously substantially bound to said first binding site; and

a second binding site to which either said second autoantibody or said non-immobilized antibody can bind;

wherein said first and second binding sites are substantially distinct sites on said antigen.

143. A kit according to claim 141, wherein said non-immobilized antibody is provided with said labelling means.

144. A kit according to claim 141, wherein said immobilized antibody comprises a first autoantibody to said antigen and said non-immobilized antibody comprises a second autoantibody to said antigen.

145. A kit according to claim 120 which further comprises a positive control that is present in the presence or absence of the at least one

autoantibody being screened, wherein the positive control comprises at least one control agent attached to the substrate that can bind to the at least one substantially non-immobilized antibody.

146. A method of screening a patient for at least one autoantibody to at least one antigen, which method comprises:

(a) obtaining a sample of body fluid from said patient;

(b) contacting said sample of body fluid of step (a) with an antigen source of a kit according to claim 115, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(c) allowing said mixture to flow relative to a substrate of said kit so as to allow said mixture to contact said antibody immobilized to said substrate; and

(d) monitoring binding of said autoantibody and said antigen present in said mixture, so as to provide an indication of the presence of said autoantibody in said sample of body fluid from said patient.

147. A method according to claim 146, for testing said patient for an autoimmune thyroid disease.

148. A method according to claim 146, which further comprises screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

149. A method of treating a patient suffering from, or susceptible to, an autoimmune disease, which method comprises:

screening said patient for at least one autoantibody to at least one antigen as defined in claim 146; and

when at least one autoantibody is detected in a sample of body fluid obtained from said patient at a level indicative of an autoimmune disease,

administering to said patient at least one therapeutically active substance effective in the treatment of the autoimmune disease.

150. A kit as defined in claim 115, and at least one therapeutically active substance effective in the treatment of an autoimmune disease.

REMARKS

The foregoing amendments add no new matter and are only added to more clearly define the invention.

The Commissioner is authorized to charge any fees or credit any overpayment in connection with the filing of this paper to our Deposit Account No. 11-1449.

It is believed this application is in condition for allowance, which allowance is respectfully requested.

Respectfully submitted,

KOHN & ASSOCIATES

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30500 Northwestern Hwy., Ste. 410
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Constance McLean

Applicant or Patentee	:	Peter John Burne et al.	Attorney's Docket No 0769.00140
Serial or Patent No.	:		
Filed or Issued	:		
For	:	Assays for Autoantibodies	

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:

[x] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN RSR Limited,

ADDRESS Avenue Park, Pentwyn, Cardiff CF2 7HE.
United Kingdom.

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed in a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, described in

[x] the specification of which was filed on 27th October 1999 as PCT patent application GB99/03548

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by an person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Bernard Rees Smith
TITLE OF PERSON OTHER THAN OWNER Director
ADDRESS OF PERSON SIGNING Richmond House
Druidstone Road
Old St. Mellons
Cardiff CF3 6XD
United Kingdom

SIGNATURE _____ DATE May 26th 2000

Applicant	:	Peter John Burne et al.	Attorney's Docket No 0769.00140
Serial or Patent No.	:		
Filed or Issued	:		
Title	:	Assays for Autoantibodies	

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled Diagnosis of Autoimmune Adrenal Disease described in

[x] the specification of which was filed on 27th October 1999 as PCT patent application GB99/03548

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e)

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[x] persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

FULL NAME RSR Limited
ADDRESS Avenue Park, Pentwyn, Cardiff CF2 7HE, United Kingdom

[ ] Individual [x] Small Business Concern [ ] Non-Profit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Bernard Rees Smith
Name of Inventor

Signature of Inventor

May 26th 2000
Date

Peter John Burne
Name of Inventor

P. J. Burne
Signature of Inventor

22/5/2000
Date

Assays for Autoantibodies

The present invention is concerned with assays for screening a sample of body fluid for autoantibodies to various antigens. In particular, the present invention is concerned with screening a sample of body fluid for autoantibodies associated with autoimmune thyroid disease.

Autoimmune diseases are characterised by the presence of circulating autoantibodies and in autoimmune thyroid disease, for example, the autoantibodies are directed to three different thyroid proteins, namely thyroid peroxidase (TPO), thyroglobulin (Tg) and the receptor for thyroid stimulating hormone (TSHR).

In the absence of thyroid disease, thyroid function is controlled by a feedback system involving the pituitary gland. The pituitary secretes the hormone thyroid stimulating hormone (TSH) into the circulating blood. TSH then acts on TSH receptors (TSHR) on the surfaces of thyroid cells in such a way as to stimulate the synthesis and release of thyroid hormones (which stimulate metabolic processes in almost all cells). As circulating thyroid hormone levels rise these hormones act back on the pituitary to inhibit TSH release. This causes blood TSH levels to fall with the effect of lowering blood thyroid hormone levels. This feedback mechanism allows circulating thyroid hormone levels to be maintained within close limits, thus ensuring good control over metabolic activity.

In thyroid disease, however, the above feedback system is often distorted. For example, when the thyroid is under-active (hypothyroidism), thyroid hormone levels are lower than normal and because these low levels do not suppress TSH release, circulating TSH levels are high. In the case of thyroid over-activity (hyperthyroidism), thyroid levels are higher than normal and these high levels cause TSH release to be suppressed more than normal and circulating TSH levels are low.

Hypothyroidism is often caused by autoimmune attack on the thyroid and this attack is associated with the formation of autoantibodies to two different thyroid proteins (autoantigens), namely TPO and Tg. Screening for autoantibodies to TPO and/or autoantibodies to Tg is important in the diagnosis and management of the various forms of autoimmune hypothyroidism, including post-partum thyroiditis and the like. This screening for TPO autoantibodies and/or Tg autoantibodies (which indicates the likely cause of thyroid underactivity) complements monitoring of circulating TSH levels or thyroid hormone levels which reflect the extent of thyroid under-activity and effectiveness of treatment.

Hyperthyroidism is also often caused by autoimmune attack on the thyroid but in this condition, autoantibodies are formed to the TSHR. These TSHR autoantibodies mimic the effects of TSH and cause circulating thyroid hormone levels to be high. Such high thyroid hormone levels act on the pituitary and suppress circulating TSH levels and

consequently TSH levels are lower than normal. Screening for autoantibodies to the TSHR is important in the diagnosis of autoimmune hyperthyroidism. As with autoimmune hypothyroidism, screening for TSHR autoantibodies complements monitoring of circulating TSH levels or thyroid hormone levels which reflect the extent of thyroid over-activity and effectiveness of treatment.

In patients with thyroid cancer, screening for circulating levels of Tg is often used as an indicator of the presence of any residual malignant thyroid tumour cells after treatment. Tg levels are usually measured by assays which depend on monoclonal and/or polyclonal antibodies to Tg but if autoantibodies to Tg are present in patient test samples, these autoantibodies can interfere with the Tg assays, giving erroneous results. Consequently, screening for autoantibodies to Tg is often carried out at the same time as detection and monitoring of circulating Tg levels.

Many examples of other (i.e. non thyroid) autoimmune diseases are known, such as type 1 diabetes (where autoantibodies are formed to insulin, glutamic acid decarboxylase and to the islet cell protein ICA512 or IA2), celiac disease (where autoantibodies are formed to tissue transglutaminase), myasthenia gravis (where autoantibodies are formed to the acetylcholine receptor and to calcium channels), systemic lupus erythematosus (where autoantibodies are formed to DNA and to various nuclear proteins), and the like.

Currently, several types of assay have been used to measure autoantibodies. These include methods using, for example, radioactive labels in which the labelled antigen binds directly to the respective autoantibody, or methods using radioactive labels in competition assays. Several non-radioactive assays have also been used, including those based on agglutination of particles coated with antigen. In addition, sandwich type enzyme linked immunosorbent assays (ELISA) are available. These sandwich type enzyme linked immunosorbent assays have used ELISA plates coated with antigen in combination with an anti-human IgG reagent conjugated with an enzyme such as horseradish peroxidase.

However, there have been some major limitations associated with current assay methods for autoantibodies, for example:-

the current assays can only be carried out away from the patient in specially equipped laboratories; and

the current assays can only be carried out by experienced personnel and take several hours to complete.

It is therefore the aim of the present invention to provide an improved assay system which alleviates some of the aforementioned problems.

It is a further object of the present invention to provide simple and rapid assay methods for the monitoring of

autoantibodies and also to provide diagnostic kits for use in the simple and rapid detection of autoantibodies for the diagnosis of autoimmune diseases. It is a further object of the present invention to provide an assay method that can be carried out near the point of patient care by personnel who do not have experience in laboratory procedures.

According to the present invention, therefore, there is provided a method of screening a sample of body fluid for at least one autoantibody to at least one antigen, which method comprises:

(a) providing a source of said at least one antigen to said autoantibody;

(b) providing a substrate having immobilised thereto at least one antibody to said antigen of step (a);

(c) contacting said antigen source of step (a) with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) allowing said mixture obtained in step (c) to flow relative to said substrate of step (b) so as to allow said mixture to contact said antibody immobilised to said substrate;

(e) providing labelling means so as to permit monitoring of binding of said autoantibody and said antigen present in said mixture obtained in step (c); and

(f) monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

A method according to the present invention is particularly suitable for use in screening for at least one autoantibody associated with autoimmune thyroid disease and where the antigen comprises a thyroid protein. Advantageously the thyroid protein is selected from the group consisting of thyroid peroxidase (TPO), thyroglobulin (Tg) and thyroid stimulating hormone receptor (TSHR), and even more advantageously the thyroid protein is selected from the group consisting of TPO and Tg.

According to a particularly preferred aspect of the present invention there is provided a method of screening a sample of body fluid for at least one autoantibody to at least one antigen comprising a thyroid protein selected from the group consisting of TPO, Tg and TSHR, which method comprises:

(a) providing a source of said at least one antigen to said autoantibody;

(b) providing a substrate having immobilised thereto at least one antibody to said antigen of step (a);

(c) contacting said antigen source of step (a) with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when

the latter is present in said sample;

(d) allowing said mixture obtained in step (c) to flow relative to said substrate of step (b) so as to allow said mixture to contact said antibody immobilised to said substrate;

(e) providing labelling means so as to permit monitoring of binding of said autoantibody and said antigen present in said mixture obtained in step (c); and

(f) monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

A method according to the present invention preferably further comprises screening, in addition to the autoantibody screening, for at least one further biological marker present in a sample of body fluid from a patient, which marker is indicative of an autoimmune (typically thyroid) disease. Suitable biological markers can be selected from the group consisting of thyroid stimulating hormone (TSH), thyroxine, tri-iodothyronine, thyroglobulin (Tg) and the like. A method according to the present invention, therefore, preferably further comprises screening for the presence of at least one of the group consisting of TSH, thyroxine, tri-iodothyronine, Tg and the like, in a sample of body fluid obtained from a patient being tested for an autoimmune thyroid disease.

Such screening for the presence of a further biological marker or markers, can in the case of at least thyroxine

and/or tri-iodothyronine, comprise screening for total or free (circulating) thyroxine and/or total or free (circulating) tri-iodothyronine. It may be desirable for means for screening for the presence of such a further biological marker or markers to be provided to the substrate employed in the present invention. Alternatively, means for screening for the presence of such a further biological marker or markers may be provided remote from the substrate substantially as hereinbefore described and suitably such remote screening means can be provided by a separate kit.

A method according to the present invention is also suitable for use in screening for at least one autoantibody associated with non-thyroid autoimmune disease, such as autoantibodies associated with type 1 diabetes, celiac disease, myasthenia gravis, systemic lupus erythematosus and the like.

It is often preferred that a method according to the present invention further employs at least one substantially non-immobilised antibody to the antigen and preferably a method according to the present invention comprises contacting in step (c) the antigen source and the sample of body fluid with the at least one substantially non-immobilised antibody. The term "substantially non-immobilised antibody" as used herein denotes an antibody that when provided in the mixture obtained in step (c) can be allowed to flow relative to the substrate employed in the present invention.

Preferably monitoring in step (f) of a method according to the present invention comprises observing a colorimetric change dependent on the binding of the autoantibody and the antigen present in the mixture obtained by step (c). Suitably the labelling means can comprise a colorimetric label selected from the group consisting of colloidal gold, colloidal carbon, coloured latex, dyed polymers and the like. Preferably the colorimetric label comprises colloidal gold. Alternatively, the monitoring in step (f) can involve electronic monitoring, whereby a visible read-out can be obtained indicative of autoantibody and antigen binding.

In the case where a colorimetric label is employed substantially as described above, the labelling means can further comprise a linker by which the colorimetric label, such as colloidal gold, may be attached to the antigen and/or antibody to be labelled. For example, a suitable linker can include -biotin-antibiotin-, -biotin-strepavidin- (SA) or the like. The labelling means may be applied to an antigen and/or antibody to be labelled substantially remote from the substrate. Alternatively, the labelling means may be provided to the substrate and such labelling means may be applied to the antigen and/or antibody to be labelled when the antigen and/or antibody has also been provided to the substrate.

Advantageously monitoring in step (f) of a method according to the present invention can further comprise providing a positive control that is present in the presence or absence

of the autoantibody or autoantibodies being screened.

For example, the positive control may comprise, attaching to the substrate, a capture reagent for a colorimetric label, such as colloidal gold or the like. Alternatively, the positive control may comprise attaching to the substrate at least one control antibody to the antigen, which control antibody binds to a site on the antigen distinct to a binding site thereof for the autoantibody or autoantibodies being screened. The control antibody is preferably attached to the substrate employed in a method according to the present invention at a location of the substrate downstream relative to the immobilised antibody. A further alternative positive control can comprise attaching to the substrate at least one control agent that can bind to the at least one substantially non-immobilised antibody. Suitably the control agent can be selected from the group consisting of anti-mouse IgG, anti-human IgG or the like, and is typically attached to the substrate downstream relative to the immobilised antibody.

Suitably, a method according to the present invention comprises allowing a mixture obtained in step (c) to flow along the substrate and interact with the antibody immobilised to the substrate. It is preferred that at least the sample of body fluid is contacted with an application zone of the substrate, which application zone is provided upstream on the substrate relative to the immobilised antibody, and wherein the mixture is allowed to flow from the application zone along the substrate so as to

interact with the immobilised antibody.

Aptly, the application zone can include the source of the antigen of step (a), and the mixture in step (c) is obtained by contacting the sample of body fluid with the antigen of the application zone. In the case where at least one substantially non-immobilised antibody is employed in a method according to the present invention, the application zone can further include the non-immobilised antibody, and the mixture in step (c) can be obtained by contacting said sample of body fluid and the antigen with the non-immobilised antibody present in the application zone.

Alternatively, the mixture of step (c) can be provided by contacting the antigen source of step (a) and the sample of body fluid substantially remote from the substrate and said mixture is subsequently contacted with the application zone of the substrate. In the case where at least one substantially non-immobilised antibody is employed in a method according to the present invention, the antigen source of step (a), the sample of body fluid and the non-immobilised antibody are contacted substantially remote from the substrate so as to provide the mixture of step (c), and the mixture is subsequently contacted with the application zone of the substrate.

In a first screening method according to the present invention, the sample of body fluid is screened for one autoantibody. Preferably the antigen includes a binding

site to which either the autoantibody or the immobilised antibody can bind, whereby in step (d) binding of the immobilised antibody to the binding site is substantially precluded where the autoantibody has substantially bound to the binding site in step (c).

In a second screening method according to the present invention, the sample of body fluid is screened for at least first and second autoantibodies to the antigen, wherein at least first and second antibodies to the antigen are immobilised to the substrate in step (b). Preferably, the antigen includes:

a first binding site to which either the first autoantibody or the first immobilised antibody can bind, whereby in step (d) binding of the first immobilised antibody to the first binding site is substantially precluded where the first autoantibody has substantially bound to the first binding site in step (c); and

a second binding site to which either the second autoantibody or the second immobilised antibody can bind, whereby in step (d) binding of the second immobilised antibody to the second binding site is substantially precluded where the second autoantibody has substantially bound to the second binding site in step (c);

wherein the first and second binding sites are

substantially distinct sites on the antigen.

Labelling means can suitably be provided to the antigen in first or second screening methods substantially as described above. Alternatively, where at least one substantially non-immobilised antibody is employed in first or second screening methods according to the present invention, the non-immobilised antibody can be provided with the labelling means, where the non-immobilised antibody is capable of binding to a site on the antigen substantially distinct from a binding site for either (i) the autoantibody or autoantibodies being screened or (ii) the immobilised antibody, whereby in step (d), antigen is allowed to be substantially bound both to the immobilised antibody and to the non-immobilised antibody.

In a third screening method according to the present invention for screening the sample of body fluid for at least first and second autoantibodies to the antigen, and where at least one substantially non-immobilised antibody is employed, the non-immobilised antibody is capable of binding to a site on the antigen to which either the first or second autoantibody can bind and which is substantially distinct to a binding site on the antigen for the immobilised antibody, whereby in step (d) antigen is allowed to be substantially bound both to the immobilised antibody and to the non-immobilised antibody.

In the third screening method according to the present invention, the antigen preferably includes:

a first binding site to which either the first autoantibody or the immobilised antibody can bind, whereby in step (d) binding of immobilised antibody to the first binding site is substantially precluded where the first autoantibody has substantially bound to the first binding site in step (c); and

a second binding site to which either the second autoantibody or the non-immobilised antibody can bind;

wherein the first and second binding sites are substantially distinct sites on the antigen.

In the third screening method according to the present invention, preferably the non-immobilised antibody is provided with the labelling means. Suitably the immobilised antibody can comprise a first autoantibody to the antigen and the non-immobilised antibody can comprise a second autoantibody to the antigen.

The present invention further provides a kit for use in screening a sample of body fluid for at least one autoantibody to at least one antigen, which kit comprises:

(a) a source of the at least one antigen to the autoantibody;

(b) a substrate having immobilised thereto at least one antibody to the antigen;

(c) means for contacting the antigen source with the sample of body fluid, so as to obtain a mixture

wherein the antigen is allowed to substantially bind with the autoantibody, when the latter is present in the sample;

(d) means for allowing the mixture to flow relative to the substrate so as to allow the mixture to contact the antibody immobilised to said substrate;

(e) labelling means to permit monitoring of binding of the autoantibody and the antigen present in the mixture; and

(f) means for monitoring the binding so as to provide an indication of the presence of the autoantibody in the sample of body fluid.

Substantially as hereinbefore described with reference to a method of screening according to the present invention, a kit according to the present invention is particularly suitable for use in screening for at least one autoantibody associated with autoimmune (typically thyroid) disease and where the antigen comprises a thyroid protein. Advantageously the thyroid protein is selected from the group consisting of thyroid peroxidase (TPO), thyroglobulin (Tg) and thyroid stimulating hormone receptor (TSHR), and even more advantageously the thyroid protein is selected from the group consisting of TPO and Tg.

According to a preferred embodiment of the present invention there is provided a kit for use in screening a sample of body fluid for at least one autoantibody to at least one antigen comprising a thyroid protein selected

from the group consisting of TPO, Tg and TSHR, which kit comprises:

(a) a source of the at least one antigen to the autoantibody;

(b) a substrate having immobilised thereto at least one antibody to the antigen;

(c) means for contacting the antigen source with the sample of body fluid, so as to obtain a mixture wherein the antigen is allowed to substantially bind with the autoantibody, when the latter is present in the sample;

(d) means for allowing the mixture to flow relative to the substrate so as to allow the mixture to contact the antibody immobilised to the substrate;

(e) labelling means to permit monitoring of binding of the autoantibody and the antigen present in the mixture; and

(f) means for monitoring the binding so as to provide an indication of the presence of the autoantibody in the sample of body fluid.

Substantially as hereinbefore described with reference to a method of screening according to the present invention a kit according to the present invention further comprises means for screening for at least one further biological marker present in patient, which marker is indicative of an autoimmune (typically thyroid) disease. Suitable biological markers can be selected from the group

consisting of thyroid stimulating hormone (TSH), thyroxine, tri-iodothyronine, thyroglobulin (Tg) and the like. Preferably, therefore, a kit according to the present invention further comprises means for screening for the presence of at least one of TSH, thyroxine, tri-iodothyronine, Tg and the like in the sample of body fluid.

Suitably, a kit according to the present invention further comprises a source of at least one substantially non-immobilised antibody to the antigen substantially as hereinbefore described and means whereby the non-immobilised antibody can be contacted with the antigen source and the sample of body fluid.

Advantageously, the monitoring means employed in a kit according to the present invention comprise means for observing a colorimetric change dependent on the binding of the autoantibody and the antigen present in said mixture substantially as hereinbefore described, although other monitoring means can be employed also substantially as hereinbefore described. Suitably the labelling means can comprise a colorimetric label, such as colloidal gold substantially as hereinbefore described and a linker can be provided for attaching the colorimetric label to an antigen and/or antibody to be labelled again substantially as hereinbefore described. In the case where labelling means are applied to an antigen and/or antibody following application thereof to the substrate, the substrate may be provided with the labelling means for subsequent application.

Preferably the substrate of a kit according to the present invention can further comprise a positive control substantially as hereinbefore described with reference to a method according to the present invention.

Preferably the substrate of a kit according to the present invention can comprise an application zone provided upstream on the substrate relative to the immobilised antibody, whereby the mixture is allowed to flow from the application zone along the substrate so as to interact with the immobilised antibody.

Suitably, that application zone can include the source of the antigen, and the mixture is obtained by contacting the sample of body fluid with the antigen of the application zone. In the case where a kit according to the present invention further comprises at least one substantially non-immobilised antibody substantially as hereinbefore described, the application zone can further include the non-immobilised antibody, and means are provided whereby the mixture is obtained by contacting the sample of body fluid and the antigen with the non-immobilised antibody present in the application zone.

Alternatively, a kit according to the present invention can comprise means whereby the antigen source and the sample of body fluid are contacted substantially remote from the substrate so as to provide the mixture and means whereby the mixture is subsequently contacted with the application zone. In the case where a kit according to the present

invention comprises at least one substantially non-immobilised antibody, means can be provided whereby the antigen source, the sample of body fluid and / or the non-immobilised antibody are contacted substantially remote from the substrate so as to provide the mixture, and means whereby the mixture is subsequently contacted with the application zone.

A kit according to the present invention preferably further comprises wick means arranged downstream relative to the immobilised antibody so as to permit or potentiate flow of at least the sample of body fluid towards the immobilised antibody.

A first kit according to the present invention is suitable for screening for one autoantibody in the sample of body fluid and the antigen includes a binding site to which either the autoantibody or the immobilised antibody can bind, whereby binding of the immobilised antibody to the binding site is substantially precluded where the autoantibody has previously substantially bound to the binding site.

A second kit according to the present invention is suitable for screening the sample of body fluid for at least first and second autoantibodies to the antigen, wherein at least first and second antibodies to the antigen are immobilised to the substrate. Preferably the antigen includes:

a first binding site to which either the first

autoantibody or the first immobilised antibody can bind, whereby binding of the first immobilised antibody to the first binding site is substantially precluded where the first autoantibody has previously substantially bound to the first binding site; and

a second binding site to which either the second autoantibody or the second immobilised antibody can bind, whereby binding of the second immobilised antibody to the second binding site is substantially precluded where the second autoantibody has previously substantially bound to the second binding site;

wherein the first and second binding sites are substantially distinct sites on the antigen.

Suitably the labelling means can be provided to the antigen of a first or second kit according to the present invention. Alternatively, where a first or second kit according to the present invention further comprises at least one substantially non-immobilised antibody, the non-immobilised antibody can be provided with the labelling means, which non-immobilised antibody is capable of binding to a site on the antigen substantially distinct from a binding site for either (i) the autoantibody or autoantibodies being screened or (ii) the immobilised antibody, whereby antigen is allowed to be substantially bound both to the immobilised antibody and to the non-immobilised antibody.

A third kit according to the present invention is suitable for screening the sample of body fluid for at least first and second autoantibodies to the antigen, which third kit further comprises at least one substantially non-immobilised antibody, wherein the non-immobilised antibody is capable of binding to a site on the antigen to which either the first or second autoantibody can bind and which is substantially distinct to a binding site on the antigen for the immobilised antibody, whereby antigen is allowed to be substantially bound both to the immobilised antibody and to the non-immobilised antibody.

Preferably the antigen of the third kit includes:

a first binding site to which either the first autoantibody or the immobilised antibody can bind, whereby binding of immobilised antibody to the first binding site is substantially precluded where the first autoantibody has previously substantially bound to the first binding site; and

a second binding site to which either the second autoantibody or the non-immobilised antibody can bind;

wherein the first and second binding sites are substantially distinct sites on the antigen.

Suitably the non-immobilised antibody is provided with the labelling means in a third kit according to the present invention. It may be preferred that the immobilised

antibody comprises a first autoantibody to the antigen and the non-immobilised antibody comprises a second autoantibody to the antigen.

Non-immobilised and immobilised antibodies employed in methods or kits according to the present invention are generally provided in substantially purified form and can comprise monoclonal antibodies, polyclonal antibodies, recombinant antibodies, antibody fragments, synthetic antibodies, substances mimicking antibodies, autoantibodies or the like. Preferred aspects of the present invention comprise the non-immobilised antibody and/or the immobilised antibody comprising an autoantibody, which may preferably be a monoclonal antibody, and/or the non-immobilised antibody and/or the immobilised antibody can comprise a monoclonal antibody.

In first and second screening methods, and first and second kits, according to the present invention , in the presence of an autoantibody or autoantibodies being screened for in a sample of body fluid, generally binding of the autoantibody or autoantibodies with the antigen precludes binding of the latter with immobilised antibody. In the case where a colorimetric label is employed in first and second screening methods and kits according to the present invention, substantially no colour change due to binding of antigen to immobilised antibody is thus seen in the presence of an autoantibody or autoantibodies; alternatively in the absence of an autoantibody or autoantibodies, a colour change is seen due to binding of

antigen to immobilised antibody.

In a third screening method and kit according to the present invention, again in the absence of autoantibody or autoantibodies being screened, a colour change is seen indicative of antigen and immobilised antibody binding. In the presence of autoantibody or autoantibodies, substantially no colour change can be seen, or some colour change can be seen due to non-immobilised antibody binding to antigen in competition with autoantibody and antigen binding whereby antigen bound to non-immobilised antibody can also bind to immobilised antibody giving a colour change.

Suitably an antigen employed in methods or kits of the present invention can comprise recombinant antigen, native antigen (autoantigen), synthetic antigen, antigen fragments, substances mimicking antigen or the like.

A substrate for use in the present invention can comprise a membrane of nitrocellulose, cellulose acetate, a polyamide or the like.

Generally the present invention comprises screening a sample of blood, plasma, serum or urine for at least one autoantibody.

The present invention further provides use of a kit substantially as hereinbefore described in screening a sample of body fluid for at least one autoantibody to at

least one antigen again substantially as hereinbefore described.

There is still further provided by the present invention a method of screening a patient for at least one autoantibody to at least one antigen, which method comprises:

(a) obtaining a sample of body fluid from the patient;

(b) contacting the sample of body fluid of step (a) with an antigen source of a kit substantially as hereinbefore described so as to obtain a mixture wherein said antigen is allowed to substantially bind with the autoantibody, when the latter is present in the sample;

(c) allowing the mixture to flow relative to a substrate of the kit of step (b) so as to allow the mixture to contact the antibody immobilised to the substrate; and

(d) monitoring binding of the autoantibody and the antigen present in the mixture, so as to provide an indication of the presence of the autoantibody in the sample of body fluid from the patient.

A method substantially as described above is preferably for testing the patient for an autoimmune thyroid disease and may preferably further comprise screening for the presence of at least one of TSH, thyroxine, tri-iodothyronine and Tg in the sample of body fluid obtained form the patient substantially as hereinbefore described.

There is further provided by the present invention a method of treating a patient suffering from, or susceptible to, an autoimmune disease, which method comprises:

screening the patient for at least one autoantibody to at least one antigen substantially as hereinbefore described; and

when at least one autoantibody is detected in a sample of body fluid obtained from the patient at a level indicative of an autoimmune disease, administering to the patient at least one therapeutically active substance effective in the treatment of the autoimmune disease.

Substantially as hereinbefore described the autoimmune disease is a thyroid autoimmune disease and the therapeutically active substance comprises a pharmaceutical effective for treatment of thyroid autoimmune disease. The mode of administration, dose and the like is generally at the discretion of an attendant physician.

There is still further provided by the present invention, in combination, a kit substantially as hereinbefore described, and at least one therapeutically active substance effective for treatment of an autoimmune disease (typically a thyroid autoimmune disease) substantially as hereinbefore described.

The present invention will now be illustrated with

reference to the accompanying figures, which are given by way of example only.

Figure 1a is a side view of a kit according to the present invention for screening TPO autoantibodies;

Figure 1b is a top view of the kit shown in Figure 1a;

Figures 2a and 2b are top views of the kit of Figures 1a and 1b and respectively show the results obtained in the absence and presence of autoantibodies to TPO in the sample of body fluid;

Figures 3a and 3b are top views of a kit according to the present invention incorporating a positive control and show the results obtained in the absence and presence of autoantibodies to TPO;

Figure 4 is a top view of a kit according to the present invention for screening for Tg autoantibodies;

Figure 5 is a top view of a kit according to the present invention for screening for both Tg autoantibodies and TPO autoantibodies;

Figure 6 is a top view of a kit for screening for autoantibodies to different parts (epitopes) of TPO;

Figure 7a is a side view of a kit according to the present invention for screening for fist and second autoantibodies

to Tg (autoantibodies Tg-AAb1 and Tg-AAb2);

Figure 7b is a top view of the kit shown in Figure 7a;

Figures 8a, 8b, 8c and 8d are top views of the kit shown in Figures 7a and 7b and show the results obtained in the absence and presence of first and/or second autoantibodies to Tg (autoantibodies Tg-AAb1 and Tg-AAb2);

Figure 9a is a side view of a kit according to the present invention for screening for first and second autoantibodies to TPO (autoantibodies TPO-AAb1 and TPO-AAb2);

Figure 9b is a top view of the kit shown in Figure 9a; and

Figures 10a and 10b are top views of the kit shown in Figures 9a and 9b and show the results obtained in the absence and presence of first and/or second autoantibodies to TPO (autoantibodies TPO-AAb1 and TPO-AAb2).

Referring firstly to Figures 1a and 1b, there is shown a kit (1) for the screening for autoantibodies to TPO comprising a zone (2) for receiving a sample of body fluid (preferably blood) and includes a cell filter (not shown) for separating red blood cells from the remainder of the blood sample. A pad (3) comprising strepavidin-gold (SA-gold) (4) is adjacent to receiving zone (2). Pad (3) is in communication with a zone comprising TPO-biotin (TPO-bi) (5) which is dried to a nitrocellulose membrane (6). Furthermore, purified antibodies to TPO (7) are immobilised

to nitrocellulose membrane (6) and are located downstream from the zone comprising TPO-bi (5). A paper wick (8) is located at an opposite end to receiving zone (2). Wick (8) is designed to permit or potentiate flow from receiving zone (2) towards purified immobilised antibodies to TPO (7).

The following series of steps illustrate the use of kit(1) shown in Figures 1a and 1b in a screening method according to the present invention.

Step 1

The first step comprises applying a sample of blood or the like to receiving zone (2). The sample of plasma will then flow towards wick (8).

Step 2

The blood cells are retained in receiving zone (2). The plasma then flows through pad (3) and forms a mixture with SA-gold (4) and this mixture flows towards TPO-bi (5).

Step 3

The plasma-SA-gold mixture arrives at the zone comprising TPO-bi (5) where TPO-bi (5) dissolves allowing formation of a TPO-bi-SA-gold complex. If autoantibodies to TPO are present in the plasma, these will bind to TPO in the TPO-bi-SA-gold complex.

Step 4

The mixture of plasma and TPO-bi-SA-gold complex then flows

towards immobilised antibodies to TPO (7).

Step 5

The reaction in the absence of autoantibodies to TPO allows the TPO-bi-SA-gold complex to bind to immobilised antibodies to TPO (7) giving a red-gold line (10), as illustrated in Figure 2a.

Step 6

The reaction in the presence of autoantibodies to TPO allows autoantibodies to TPO in the plasma to bind to TPO-bi-SA-gold complex preventing the complex from binding to immobilised TPO antibodies (7). Therefore, the absence of a red-gold line at the site of immobilised TPO antibodies (7) indicates the presence of TPO autoantibodies in the sample of body fluid.

Referring now to Figure 3a, there can be seen an embodiment of the present invention which is extended to provide a positive control which is stained red irrespective of the presence or absence of TPO autoantibodies. A rabbit antibody (11) to TPO is stained red-gold by a TPO-bi-SA-gold complex giving a red-gold line (12). Therefore, as illustrated, in the absence of autoantibodies to TPO in the sample of body fluid to be tested, immobilised antibodies to TPO (7) are stained red-gold by the TPO-bi-SA-gold complex giving red-gold line (10) (which is also illustrated in Figure 2a). Therefore, two red-gold lines (10, 12) will be indicative of a sample of body fluid which does not contain autoantibodies to TPO.

Figure 3b is identical to Figure 3a except that it illustrates a reaction where the sample of body fluid to be tested comprises autoantibodies to TPO. In this situation, autoantibodies to TPO in plasma bind to a TPO-bi-SA-gold complex thereby preventing the complex binding to immobilised antibodies to TPO (7). Therefore, in this case, only one red-gold line (for the control)namely line (12), will be visible indicating the presence of TPO autoantibodies in the sample of body fluid.

Figure 4 illustrates a kit (13) for screening for Tg autoantibodies. Kit (13) is identical to kit (1) as shown in Figure 1, apart from the replacement of TPO-bi with Tg-bi and replacement of the immobilised antibodies to TPO with immobilised antibodies to Tg. More particularly, kit (13) similarly comprises receiving zone (2), pad (3) comprising SA-gold (4) and wick (8). Kit (13) further comprises a zone comprising Tg-bi (14) dried to nitrocellulose membrane (6). Purified antibodies to Tg (15) are immobilised to nitrocellulose membrane (6) and located downstream from Tg-bi (14). An immobilised rabbit antibody to Tg (16) is also present to provide a positive control.

Figure 5 illustrates a single kit (17) for screening for both autoantibodies to TPO and autoantibodies to Tg. More particularly, kit (17) comprises (as previously referred to in kits (1) and (13) receiving zone (2), pad (3) comprising SA-gold (4) and wick (8). Kit (17) further comprises a

zone including both TPO-bi (5) and Tg-bi (14) dried to nitrocellulose membrane (6). Purified antibodies to TPO (7) and purified antibodies to Tg (15) are immobilised to nitrocellulose membrane (6) and are located downstream from TPO-bi (5) and Tg-bi (14). Immobilised rabbit antibodies to TPO (11) and Tg (16) are also present to provide a positive control.

Figure 6 illustrates a kit (18) for screening for autoantibodies to different parts (first and second epitopes) of TPO. This kit comprises immobilised antibodies to different autoantigenic epitopes on TPO which are used for the detection of autoantibodies to different parts (first and second epitopes) of TPO. More particularly, kit (18) comprises [as previously referred to in Kits (1), (13) and (17)] receiving zone (2), pad (3) comprises SA-gold (4) and wick (8). TPO-bi (5) is dried to nitrocellulose membrane (6).

TPO antibodies to the first epitope (19) and TPO antibodies to the second epitope (20) are immobilised on nitrocellulose membrane (6). An immobilised rabbit antibody to TPO (11) is also present to provide a positive control.

Referring to Figures 7a and 7b, there is shown a kit (21) for screening for first and second autoantibodies to Tg (autoantibodies Tg-AAb1 and Tg-AAb2). As previously referred to in kits (1), (13), (17) and (18), kit (21) comprises zone (2) for receiving a sample of body fluid,

pad (3), nitrocellulose membrane (6) and wick (8). Tg-gold (22) is present on pad (3) and is provided adjacent to zone (2). Tg-gold denotes Tg previously labelled with -biotin-antibiotin-colloidal gold and subsequently applied to pad (3).

Purified first and second antibodies (15a, 15b) are immobilised (immobilised antibodies Tg-Ab1 and Tg-Ab2) to nitrocellulose membrane (6) and are located downstream from Tg-gold (22). An immobilised rabbit antibody to Tg (16) is also present to provide a positive control.

Autoantibody Tg-AAb1 binds to the same site of Tg-gold (22) as immobilised Tg-Ab1 (15a). Autoantibody Tg-AAb2 binds to the same site of Tg-gold (22) as immobilised Tg-Ab2 (15b).

The following series of steps illustrate the use of kit (21) shown in Figures 7a and 7b in a screening method according to the present invention.

Step 1

The first step comprises applying a sample of blood or the like to receiving zone (2). The sample of plasma will then flow towards wick (8).

Step 2

The blood cells are retained in receiving zone (2). The plasma then flows through pad (3) and forms a mixture with Tg-gold (22) and this mixture flows towards immobilised antibodies Tg-Ab1 and Tg-Ab2 (15a, 15b).

Step 3

The mixture of plasma and Tg-gold (22) reaches immobilised antibodies Tg-Ab1 and Tg-Ab2 (15a, 15b).

Step 4

The reaction in the absence of autoantibodies Tg-AAb1 and Tg-AAb2 to Tg in the plasma allows Tg-gold (22) to bind to both immobilised antibodies Tg-Ab1 and Tg-Ab2 (15a, 15b), giving two red gold lines (23, 24) in addition to control line (25) for Tg rabbit antibody (16), as illustrated in Figure 8a.

Step 5

The reaction in the presence of autoantibody Tg-AAb1 to Tg in the plasma allows autoantibody Tg-AAb1 in the plasma to bind to Tg-gold (22) preventing Tg-gold (22) from binding to immobilised antibody Tg-Ab1 (15a). No red gold line is seen at the site of immobilised antibody Tg-Ab1 and indicates the presence of autoantibody Tg-AAb1 to Tg in the plasma. This reaction in the absence of autoantibody Tg-AAb2 to Tg in the plasma allows Tg-gold (22) to bind to immobilised antibody Tg-Ab2 (15b) and a red gold line (24) is seen in addition to control line (25), as illustrated in Figure 8b.

Step 6

The reaction in the presence of autoantibody Tg-AAb2 to Tg in the plasma allows autoantibody Tg-AAb2 in the plasma to bind to Tg-gold (22) preventing Tg-gold (22) from binding to immobilised antibody Tg-Ab2 (15b). No red gold line is

seen at the site of immobilised antibody Tg-Ab2 (15b) and this indicates the presence of autoantibody Tg-AAb2 to Tg in the plasma. This reaction in the absence of autoantibody Tg-AAb1 to Tg in the plasma allows Tg-gold (22) to bind to immobilised antibody Tg-Ab1 (15a) and a red gold line (23) is seen in addition to control line (25), as illustrated in Figure 8c.

Step 7

The reaction in the presence of both autoantibodies Tg-AAb1 and Tg-AAb2 to Tg in the plasma allows autoantibodies Tg-AAb1 and Tg-AAb2 in the plasma to bind the Tg-gold (22). Tg-gold (22) is prevented from binding to immobilised antibodies Tg-Ab1 and Tg-Ab2 (15a, 15b). No red gold lines are seen at the sites of immobilised antibodies Tg-Ab1 and Tg-Ab2 (15a, 15b), indicating the presence of autoantibodies Tg-AAb1 and Tg-AAb2 to Tg in the plasma. Red gold control line (25) is seen, as illustrated in Figure 8d.

Referring to Figures 9a and 9b, there is shown a kit (26) for the screening of first and second autoantibodies to TPO (autoantibodies TPO-AAb1 and TPO-AAb2 respectively). As previously referred to in kits (10, (13), (17), (18) and (21), kit (26) comprises zone (2) for receiving a sample of body fluid, pad (3), nitrocellulose membrane (6) and wick (8). Non-immobilised first antibody to TPO, labelled with colloidal gold, (non-immobilised antibody TPO-Ab1-gold) (27), is provided on pad (3) and is provided adjacent to zone (2). Non-immobilised antibody TPO-Ab1-gold (27)

denotes non-immobilised antibody TPO-Ab1 previously labelled with -biotin-antibiotin-colloidal gold and subsequently applied to pad (3). TPO (28) is dried to nitrocellulose membrane (6). Purified second antibody to TPO (29) is immobilised (immobilised antibody TPO-Ab2) to nitrocellulose membrane (6) and is located downstream of non-immobilised antibody TPO-Ab1-gold (27) and TPO (28).

Non-immobilised antibody TPO-Ab1-gold binds to the same site of TPO (28) as autoantibody TPO-AAb1. Immobilised antibody TPO-Ab2 binds to the same site of TPO (28) as autoantibody TPO-AAb2.

The following series of steps illustrate the use of kit (26) shown in Figures 9a and 9b in a screening method according to the present invention.

Step 1

The first step comprises applying a sample of blood or the like to receiving zone (2). The sample will then flow towards wick (8).

Step 2

The blood cells are retained in receiving zone (2). The plasma then flows through pad (3) and forms a mixture with non-immobilised antibody TPO-Ab1-gold (27) and this mixture flows towards TPO (28).

Step 3

The mixture of step 2 reaches TPO (28) and TPO (28) also

dissolves in the mixture.

Step 4

Non-immobilised antibody TPO-Ab1-gold (27) binds with TPO (28) in the mixture, unless autoantibody TPO-AAb1 is present in the plasma. In the presence of autoantibody TPO-AAb1 in the plasma, autoantibody TPO-AAb1 and non-immobilised antibody TPO-Ab1-gold (27) compete for binding with TPO (28) in the mixture.

Step 5

The mixture of plasma, non-immobilised antibody TPO-Ab1-gold (27) and TPO (28) reach immobilised antibody TPO-Ab2 (29).

Step 6

The reaction in the absence of autoantibodies TPO-AAb1 and TPO-AAb2 to TPO in the plasma allows TPO (28) to bind to non-immobilised antibody TPO-Ab1-gold (27) and immobilised antibody TPO-Ab2 (29). A red gold line (30) at the location of immobilised antibody TPO-Ab2 (29) indicates the absence of autoantibodies TPO-AAb1 and TPO-AAb2 in the plasma, as illustrated in Figure 10a.

Step 7

The reaction in the presence of autoantibody TPO-AAb1 and / or autoantibody TPO-AAb2 in the plasma sets up a competition reaction wherein binding of autoantibodies TPO-AAb1 and/or TPO-AAb2 competes with binding of non-immobilised antibody TPO-Ab1-gold (27) and immobilised

antibody TPO-Ab2 (29) with TPO (28). In the case where autoantibodies TPO-AAb1 and / or TPO-AAb2 prevent binding of TPO (28) with non-immobilised antibody TPO-Ab1-gold (27) and / or immobilised antibody TPO-Ab2 (29), no red gold line is seen as illustrated in Figure 10b. Alternatively, where competition exists as above in the presence of autoantibodies TPO-AAb1 and TPO-AAb2, there may still be some binding of TPO (28) with non-immobilised antibody TPO-Ab1-gold (27) and immobilised antibody TPO-Ab2 (29), but such binding will be to a lesser extent compared to that of step 6, and a qualitative measure of the quantity of autoantibodies TPO-AAb1 and TPO-AAb2 in the sample will be obtained.

The present invention will now be further illustrated by the following Examples which do not limit the scope of the invention in any way.

Example 1

The following tables give the results obtained using a TPO screening kit as shown in Figures 1a and 1b and a Tg screening kit as shown in Figure 4.

Table 1

Patient sample results - TPOAb

TPOAb rapid assay

Patient sample	TPOAb concentration by radioactive assay (U/ml)	Qualitative result by rapid assay
+TPOAb		
1	165.6 (+)	+
2	19.0 (+)	+
3	14.6 (+)	+
4	56.6 (+)	+
5	50.1 (+)	+
6	108.7 (+)	+
7	171.4 (+)	+
8	90.7 (+)	+
-TPOAb		
9	0.2 (-)	-
10	0.1 (-)	-
11	0.2 (-)	-
12	0.2 (-)	-
13	0.3 (-)	-
14	0.3 (-)	-

Table 2

Patient sample results - TgAb

TgAb rapid assay

Patient sample	TgAb concentration by radioactive assay (U/ml)	Qualitative result by rapid assay
+TgAb		
1	52.4 (+)	+
2	45.3 (+)	+
-TgAb		
3	Neg	-
4	Neg	-

Example 2

This Example describes screening for autoantibodies to Tg using a kit as illustrated in Figures 7 and 8.

90 μl of plasma (or sera) or 30 μl of whole blood plus 60 μl of a diluent buffer (150 mM NaCl, 20 mM Tris pH7.6) were used. Results were obtained after 10 minutes. Prior art reference radioactive method was based on that of Beever et al Clinical Chemistry 35 (1989) 1949-1954.

Table 3

(a) Results obtained in whole blood or plasma obtained from 30 healthy blood donors.

	plasma (n=30)	whole blood (Reference method cannot be used with whole blood)
Tg autoantibody positive by reference radioactive test	3/30	-
Tg autoantibody positive by a method according to the present invention employing a kit as illustrated in figures 7 and 8	3/30	1/30
Tg autoantibody negative by reference radioactive test	27/30	-
Tg autoantibody negative by a method according to the present invention employing a kit as illustrated in figures 7 and 8	27/30	29/30

Table 4

(b) Results obtained with sera from patients with systemic lupus erythematosus

	Systemic lupus erythematosus
Tg autoantibody positive by reference radioactive test	3/10
Tg autoantibody positive by a method according to the present invention employing a kit as illustrated in figures 7 and 8	3/10
Tg autoantibody negative by reference radioactive test	7/10
Tg autoantibody negative by a method according to the present invention employing a kit as illustrated in figures 7 and 8	7/10

The above data shows that a method for screening Tg autoantibodies according to the present invention can detect Tg autoantibodies in plasma from healthy blood donors, or sera from patients with systemic lupus erythematosus, at the same prevalence as the reference radioactive test.

Example 3

The Example describes screening for autoantibodies to TPO using a kit as illustrated in Figures 9 and 10.

90 μl of plasma (or sera) or 30 μl of whole blood plus 60 μl of a diluent buffer (150 mM Nacl; 20 mM Tris pH 7.6) were used. Results were obtained after 10 minutes. Prior art reference radioactive method was based on that of Beever et al Clinical Chemistry 35 (1989) 1949-1954.

Table 5

(a) Results obtained in whole blood or plasma obtained from 30 healthy blood donors

	plasma	whole blood (reference method cannot be used with whole blood)
TPO autoantibody positive by reference radioactive test	3/30	-
TPO autoantibody positive by a method according to the present invention employing a kit as illustrated in Figures 9 and 10	3/30	3/30
TPO autoantibody negative by reference radioactive test	27/30	-
TPO autoantibody negative by a method according to the present invention employing a kit as illustrated in Figures 9 and 10	27/30	27/30

Table 6

(b) Results obtained in sera from patients with suspected autoimmune thyroid disease

	Suspected autoimmune thyroid disease
TPO autoantibody positive by reference radioactive test	10/13
TPO autoantibody positive by a method according to the present invention employing a kit as illustrated in figures 9 and 10	10/13
TPO autoantibody negative by reference radioactive test	3/13
TPO autoantibody negative by a method according to the present invention employing a kit as illustrated in figures 9 and 10	3/13

The above data indicates that a method for screening TPO autoantibodies according to the present invention can detect TPO autoantibodies in (a) healthy blood donors or (b) patients suspected of having autoimmune thyroid disease at the same prevalence as the reference radioactive test.

CLAIMS:

1. A method of screening a sample of body fluid for at least one autoantibody to at least one antigen, which method comprises:

   (a) providing a source of said at least one antigen to said autoantibody;

   (b) providing a substrate having immobilised thereto at least one antibody to said antigen of step (a);

   (c) contacting said antigen source of step (a) with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

   (d) allowing said mixture obtained in step (c) to flow relative to said substrate of step (b) so as to allow said mixture to contact said antibody immobilised to said substrate;

   (e) providing labelling means so as to permit monitoring of binding of said autoantibody and said antigen present in said mixture obtained in step (c); and

   (f) monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

2. A method according to claim 1, wherein said antigen comprises a thyroid protein.

3. A method according to claim 2, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase, thyroglobulin and thyroid stimulating hormone receptor.

4. A method according to claim 3, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase and thyroglobulin.

5. A method of screening a sample of body fluid for at least one autoantibody to at least one antigen comprising a thyroid protein selected from the group consisting of thyroid peroxidase, thyroglobulin and thyroid stimulating hormone receptor, which method comprises:

(a) providing a source of said at least one antigen to said autoantibody;

(b) providing a substrate having immobilised thereto at least one antibody to said antigen of step (a);

(c) contacting said antigen source of step (a) with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) allowing said mixture obtained in step (c) to flow relative to said substrate of step (b) so as to allow said mixture to contact said antibody

immobilised to said substrate;

(e) providing labelling means so as to permit monitoring of binding of said autoantibody and said antigen present in said mixture obtained in step (c); and

(f) monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

6. A method according to claim 5, wherein said thyroid protein is thyroid peroxidase or thyroglobulin.

7. A method according to any of claims 1 to 6, which further comprises screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

8. A method according to any preceding claim, which comprises contacting in step (c) said antigen source and said sample of body fluid with at least one substantially non-immobilised antibody to said antigen.

9. A method according to claim 8, wherein said non-immobilised antibody is provided in substantially purified form.

10. A method according to claim 8 or 9, wherein said non-immobilised antibody comprises a monoclonal antibody.

11. A method according to any of claims 8 to 10, wherein said non-immobilised antibody comprises an autoantibody to said antigen.

12. A method according to any preceding claim, wherein said monitoring in step (f) comprises observing a colorimetric change dependent on said binding of said autoantibody and said antigen present in said mixture of step (c).

13. A method according to claim 12, wherein said labelling means include colloidal gold.

14. A method according to any preceding claim, which further comprises providing a positive control that is present in the presence or absence of the autoantibody or autoantibodies being screened.

15. A method according to any preceding claim, wherein said mixture obtained in step (c) is allowed to flow along said substrate and interact with said antibody immobilised to said substrate.

16. A method according to claim 15, wherein at least said sample of body fluid is contacted with an application zone of said substrate, which application zone is provided upstream on said substrate relative to said immobilised antibody, and wherein said mixture is allowed to flow from said application zone along said

substrate so as to interact with said immobilised antibody.

17. A method according to claim 16, wherein said application zone includes said source of said antigen of step (a), and said mixture in step (c) is obtained by contacting said sample of body fluid with said antigen of said application zone.

18. A method according to claim 16 or 17 as dependent on any of claims 8 to 11, wherein said application zone further includes said non-immobilised antibody, and said mixture in step (c) is obtained by contacting said sample of body fluid and said antigen with said non-immobilised antibody present in said application zone.

19. A method according to claim 16, wherein said antigen source of step (a) and said sample of body fluid are contacted substantially remote from said substrate so as to provide said mixture of step (c), and said mixture is subsequently contacted with said application zone.

20. A method according to claim 19 as dependent on any of claims 8 to 11, wherein said antigen source of step (a), said sample of body fluid and/or said non-immobilised antibody are contacted substantially remote from said substrate so as to provide said mixture of step (c), and said mixture is subsequently

contacted with said application zone.

21. A method according to any preceding claim, wherein said substrate comprises a membrane of nitrocellulose, cellulose acetate or a polyamide.

22. A method according to any preceding claim, wherein said immobilised antibody is in substantially purified form.

23. A method according to any preceding claim, wherein said immobilised antibody comprises an autoantibody to said antigen.

24. A method according to any of claims 1 to 23, wherein said immobilised antibody comprises a monoclonal antibody.

25. A method according to any preceding claim, wherein said sample of body fluid comprises blood, plasma, serum or urine.

26. A method according to any preceding claim, which comprises screening said sample of body fluid for one said autoantibody.

27. A method according to claim 26, wherein said antigen includes a binding site to which either said autoantibody or said immobilised antibody can bind, whereby in step (d) binding of said immobilised

antibody to said binding site is substantially precluded where said autoantibody has substantially bound to said binding site in step (c).

28. A method according to any of claims 1 to 25, which comprises screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein at least first and second antibodies to said antigen are immobilised on said substrate in step (b).

29. A method according to claim 28, wherein said antigen includes:

a first binding site to which either said first autoantibody or said first immobilised antibody can bind, whereby in step (d) binding of said first immobilised antibody to said first binding site is substantially precluded where said first autoantibody has substantially bound to said first binding site in step (c); and

a second binding site to which either said second autoantibody or said second immobilised antibody can bind, whereby in step (d) binding of said second immobilised antibody to said second binding site is substantially precluded where said second autoantibody has substantially bound to said second binding site in step (c);

wherein said first and second binding sites are

substantially distinct sites on said antigen.

30. A method according to any of claims 26 to 29, wherein said antigen is provided with said labelling means.

31. A method according to any of claims 26 to 30, as dependent on claim 14, wherein said positive control comprises attaching to the substrate at least one control antibody to the antigen, which control antibody binds to a site on the antigen distinct to a binding site thereof for the autoantibody or autoantibodies being screened.

32. A method according to any of claims 26 to 29 as dependent on any of claims 8 to 11, wherein said non-immobilised antibody is provided with said labelling means, which non-immobilised antibody is capable of binding to a site on said antigen substantially distinct from a binding site for either (i) said autoantibody or autoantibodies being screened or (ii) said immobilised antibody, whereby in step (d), antigen is allowed to be substantially bound both to said immobilised antibody and to said non-immobilised antibody.

33. A method according to any of claims 8 to 25 as dependent on any of claims 8 to 11, which comprises screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein said non-immobilised antibody is capable of binding to

a site on said antigen to which either said first or second autoantibody can bind and which is substantially distinct to a binding site on said antigen for said immobilised antibody, whereby in step (d) antigen is allowed to be substantially bound both to said immobilised antibody and to said non-immobilised antibody.

34. A method according to claim 33, wherein said antigen includes:

a first binding site to which either said first autoantibody or said immobilised antibody can bind, whereby in step (d) binding of immobilised antibody to said first binding site is substantially precluded where said first autoantibody has substantially bound to said first binding site in step (c); and

a second binding site to which either said second autoantibody or said non-immobilised antibody can bind;

wherein said first and second binding sites are substantially distinct sites on said antigen.

35. A method according to claim 33 or 34, wherein said non-immobilised antibody is provided with said labelling means.

36. A method according to claims 33 to 35 as dependent on claims 11 and 23, wherein said immobilised antibody comprises a first autoantibody to said antigen and said non-immobilised antibody comprises a second autoantibody to said antigen.

37. A method according to any of claims 32 to 36, wherein the positive control comprises attaching to the substrate at least one control agent that can bind to the at least one substantially non-immobilised antibody.

38. A kit for use in screening a sample of body fluid for at least one autoantibody to at least one antigen, which kit comprises:

(a) a source of said at least one antigen to said autoantibody;

(b) a substrate having immobilised thereto at least one antibody to said antigen;

(c) means for contacting said antigen source with said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) means for allowing said mixture to flow relative to said substrate so as to allow said mixture to contact said antibody immobilised to said substrate;

(e) labelling means to permit monitoring of binding

of said autoantibody and said antigen present in said mixture; and

(f) means for monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

39. A kit according to claim 38, wherein said antigen comprises a thyroid protein.

40. A kit according to claim 39, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase, thyroglobulin and thyroid stimulating hormone receptor.

41. A kit according to claim 40, wherein said thyroid protein is selected from the group consisting of thyroid peroxidase and thyroglobulin.

42. A kit for use in screening a sample of body fluid for at least one autoantibody to at least one antigen comprising a thyroid protein selected from the group consisting of thyroid peroxidase, thyroglobulin and thyroid stimulating hormone receptor, which kit comprises:

(a) a source of said at least one antigen to said autoantibody;

(b) a substrate having immobilised thereto at least one antibody to said antigen;

(c) means for contacting said antigen source with

said sample of body fluid, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(d) means for allowing said mixture to flow relative to said substrate so as to allow said mixture to contact said antibody immobilised to said substrate;

(e) labelling means to permit monitoring of binding of said autoantibody and said antigen present in said mixture; and

(f) means for monitoring said binding so as to provide an indication of the presence of said autoantibody in said sample of body fluid.

43. A kit according to claim 42, wherein said thyroid protein is thyroid peroxidase or thyroglobulin.

44. A kit according to any of claims 38 to 43, which further comprises means for screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

45. A kit according to any of claims 38 to 44, which further comprises a source of at least one substantially non-immobilised antibody to said antigen and means whereby said non-immobilised antibody can be contacted with said antigen source and said sample of body fluid.

46. A kit according to claim 45, wherein said non-immobilised antibody is provided in substantially purified form.

47. A kit according to claim 45 or 46, wherein said non-immobilised antibody comprises a monoclonal antibody.

48. A kit according to any of claims 45 to 47, wherein said non-immobilised antibody comprises an autoantibody to said antigen.

49. A kit according to any of claims 38 to 48, wherein said monitoring means comprise means for observing a colorimetric change dependent on said binding of said autoantibody and said antigen present in said mixture.

50. A kit according to claim 49, wherein said labelling means include colloidal gold.

51. A kit according to any of claims 38 to 50, which further comprises a positive control that is present in the presence or absence of the autoantibody being screened.

52. A kit according to any of claims 38 to 51, wherein said substrate comprises an application zone for at least said sample of body fluid, which application zone is provided upstream on said substrate relative to said immobilised antibody, whereby said mixture is

allowed to flow from said application zone along said substrate so as to interact with said immobilised antibody.

53. A kit according to claim 52, wherein said application zone includes said source of said antigen, and said mixture is obtained by contacting said sample of body fluid with said antigen of said application zone.

54. A kit according to claim 52 or 53 as dependent on any of claims 45 to 48, wherein said application zone further includes said non-immobilised antibody, and means whereby said mixture is obtained by contacting said sample of body fluid and said antigen with said non-immobilised antibody present in said application zone.

55. A kit according to claim 52, wherein means are provided whereby said antigen source and said sample of body fluid are contacted substantially remote from said substrate so as to provide said mixture and means whereby said mixture is subsequently contacted with said application zone.

56. A kit according to claim 55 as dependent on any of claims 45 to 48, wherein means are provided whereby said antigen source, said sample of body fluid and/or said non-immobilised antibody are contacted substantially remote from said substrate so as to provide said mixture, and means whereby said mixture

is subsequently contacted with said application zone.

57. A kit according to any of claims 38 to 56, wherein said substrate comprises a membrane of nitrocellulose, cellulose acetate or a polyamide.

58. A kit according to any of claims 38 to 57, wherein said immobilised antibody is provided in substantially purified form.

59. A kit according to any of claims 38 to 58, wherein said immobilised antibody comprises an autoantibody to said antigen.

60. A kit according to any of claims 38 to 59, wherein said immobilised antibody comprises a monoclonal antibody.

61. A kit according to any of claims 38 to 60, wherein said sample of body fluid comprises blood, plasma, serum or urine.

62. A kit according to any of claims 38 to 61, for screening said sample of body fluid for one said autoantibody, wherein said antigen includes a binding site to which either said autoantibody or said immobilised antibody can bind, whereby binding of said immobilised antibody to said binding site is substantially precluded where said autoantibody has previously substantially bound to said binding site.

63. A kit according to any of claims 38 to 61, for screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein at least first and second antibodies to said antigen are immobilised on said substrate.

64. A kit according to claim 63, wherein said antigen includes:

a first binding site to which either said first autoantibody or said first immobilised antibody can bind, whereby binding of said first immobilised antibody to said first binding site is substantially precluded where said first autoantibody has previously substantially bound to said first binding site; and

a second binding site to which either said second autoantibody or said second immobilised antibody can bind, whereby binding of said second immobilised antibody to said second binding site is substantially precluded where said second autoantibody has previously substantially bound to said second binding site;

wherein said first and second binding sites are substantially distinct sites on the antigen.

65. A kit according to any of claims 61 to 64, wherein

said antigen is provided with said labelling means.

66. A kit according to any of claims 62 to 65, as dependent on claim 51, wherein the positive control comprises attaching to the substrate at least one control antibody to the antigen, which control antibody binds to a site on the antigen distinct to a binding site thereof for the autoantibody or autoantibodies being screened.

67. A kit according to any of claims 62 to 64, as dependent on any of claims 45 to 48, wherein said non-immobilised antibody is provided with said labelling means, which non-immobilised antibody is capable of binding to a site on said antigen substantially distinct from a binding site for either (i) said autoantibody or autoantibodies being screened or (ii) said immobilised antibody, whereby antigen is allowed to be substantially bound both to said immobilised antibody and to said non-immobilised antibody.

68. A kit according to any of claims 38 to 61, as dependent on any of claims 45 to 48 for screening said sample of body fluid for at least first and second autoantibodies to said antigen, wherein said non-immobilised antibody is capable of binding to a site on said antigen to which either said first or second autoantibody can bind and which is substantially distinct to a binding site on said antigen for said immobilised antibody, whereby antigen is allowed to be

substantially bound both to said immobilised antibody and to said non-immobilised antibody.

69. A kit according to claim 68, wherein said antigen includes:

a first binding site to which either said first autoantibody or said immobilised antibody can bind, whereby binding of immobilised antibody to said first binding site is substantially precluded where said first autoantibody has previously substantially bound to said first binding site; and

a second binding site to which either said second autoantibody or said non-immobilised antibody can bind;

wherein said first and second binding sites are substantially distinct sites on said antigen.

70. A kit according to claim 68 or 69, wherein said non-immobilised antibody is provided with said labelling means.

71. A kit according to any of claims 68 to 70, as dependent on claims 48 and 59, wherein said immobilised antibody comprises a first autoantibody to said antigen and said non-immobilised antibody comprises a second autoantibody to said antigen.

72. A kit according to any of claims 67 to 71, as dependent on claim 51, wherein the positive control comprises attaching to the substrate at least one control agent that can bind to the at least one substantially non-immobilised antibody.

73. Use of a kit according to any of claims 38 to 72, in screening a sample of body fluid for at least one autoantibody to at least one antigen.

74. A method of screening a patient for at least one autoantibody to at least one antigen, which method comprises:

(a) obtaining a sample of body fluid from said patient;

(b) contacting said sample of body fluid of step (a) with an antigen source of a kit according to any of claims 38 to 72, so as to obtain a mixture wherein said antigen is allowed to substantially bind with said autoantibody, when the latter is present in said sample;

(c) allowing said mixture to flow relative to a substrate of a kit according to any of claims 38 to 72, so as to allow said mixture to contact said antibody immobilised to said substrate; and

(d) monitoring binding of said autoantibody and said antigen present in said mixture, so as to provide an indication of the presence of said

autoantibody in said sample of body fluid from said patient.

75. A method according to claim 74, for testing said patient for an autoimmune thyroid disease.

76. A method according to claim 74 or 75, which further comprises screening for the presence of at least one of thyroid stimulating hormone, thyroxine, tri-iodothyronine and thyroglobulin in said sample of body fluid.

77. A method of treating a patient suffering from, or susceptible to, an autoimmune disease, which method comprises:

screening said patient for at least one autoantibody to at least one antigen as defined in any of claims 74 to 76; and

when at least one autoantibody is detected in a sample of body fluid obtained from said patient at a level indicative of an autoimmune disease, administering to said patient at least one therapeutically active substance effective in the treatment of the autoimmune disease.

78. In combination, a kit as defined in any of claims 38 to 72, and at least one therapeutically active substance effective in the treatment of an autoimmune

disease.

79. A method substantially as hereinbefore described, substantially as described in one of the Examples.

80. A kit substantially as hereinbefore described, substantially as described in one of the Examples.

81. A kit substantially as hereinbefore described, substantially as illustrated in one or more of Figures 1a, 1b, 2a, 2b, 3a, 3b, 4, 5, 6, 7a, 7b, 8a, 8b, 8c, 8d, 9a, 9b, 10a or 10b.

Fig 1a

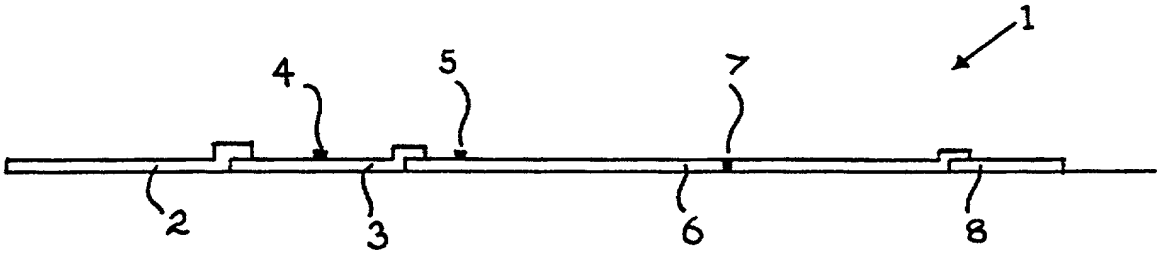


Fig 1b

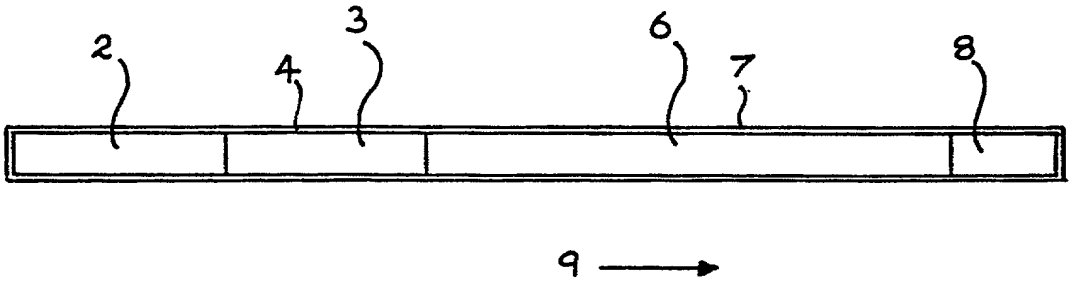


Fig 2a

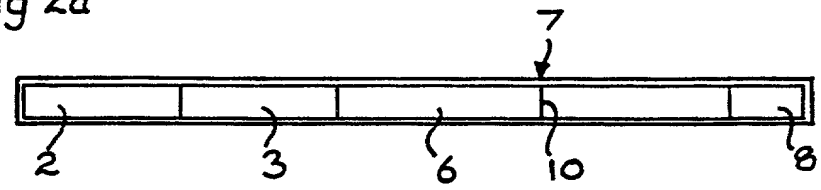


Fig 2b

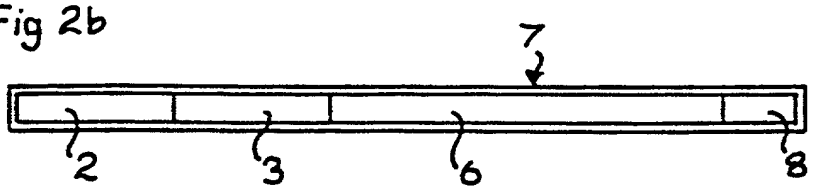


Fig 3a

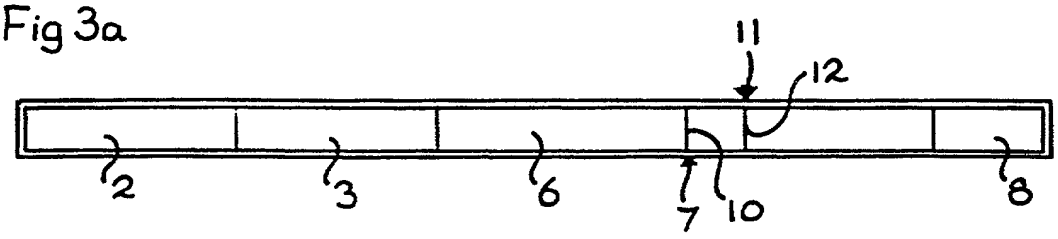


Fig 3b

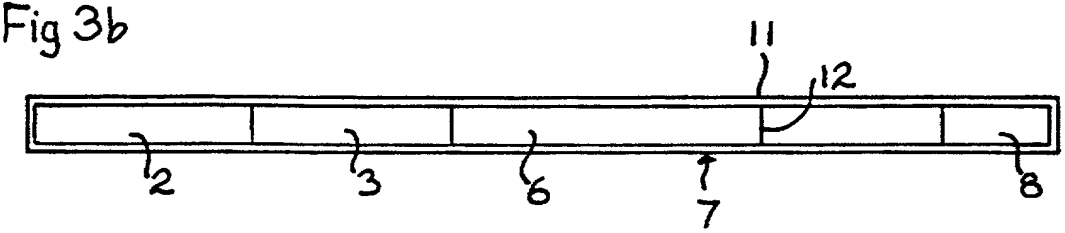


Fig 4

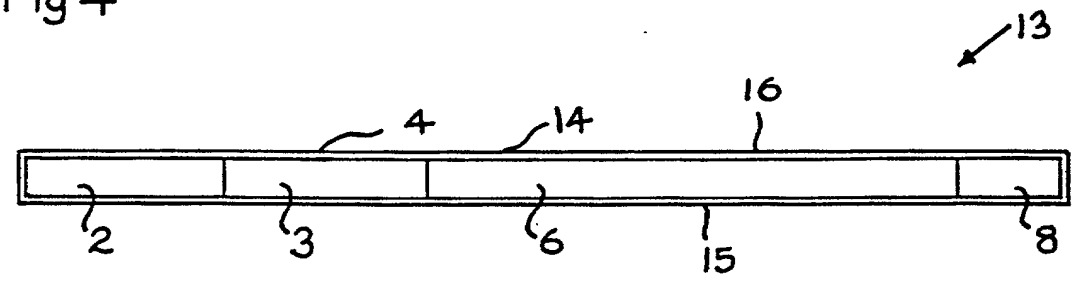


Fig 5

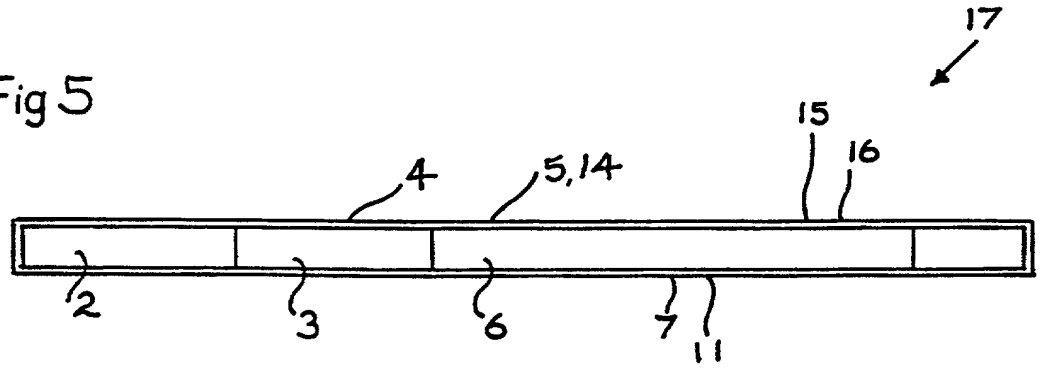


Fig 6

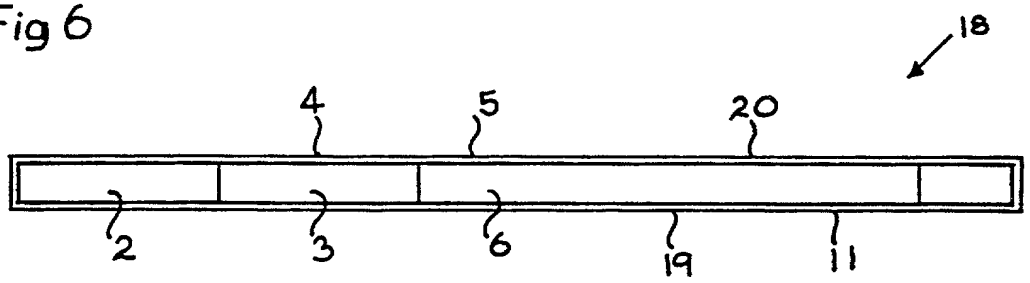


Fig 7a

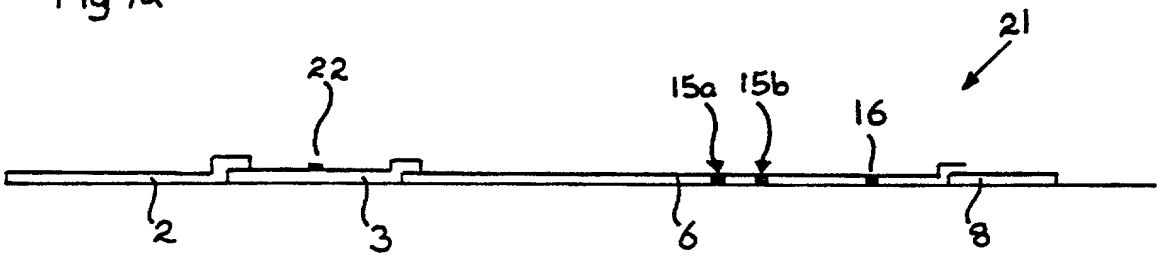


Fig 7b

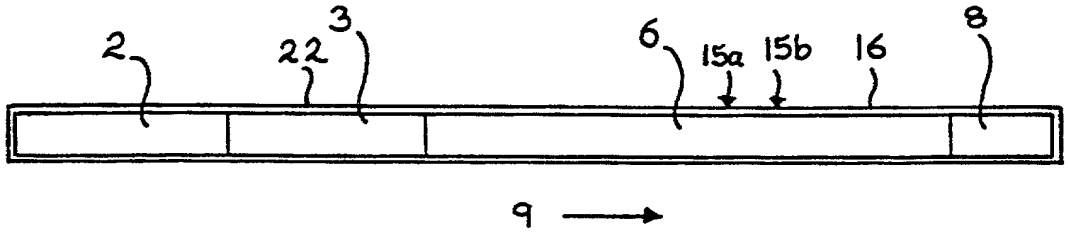


Fig 8a

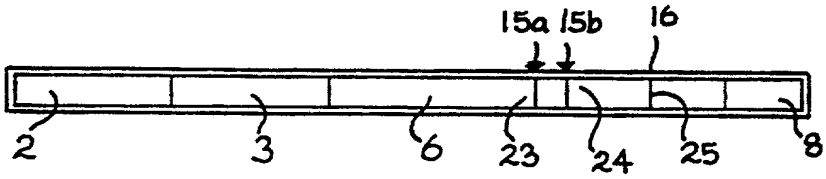


Fig 8b

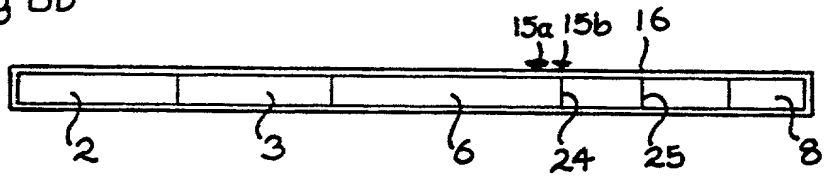


Fig 8c

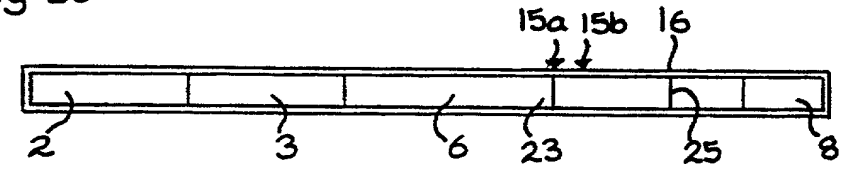


Fig 8d

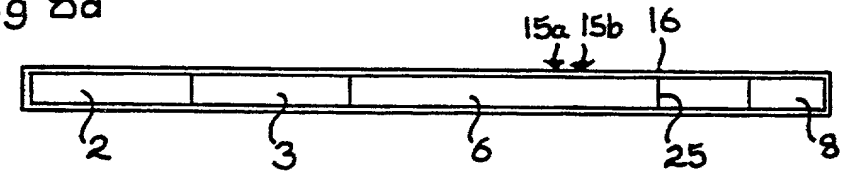


Fig 9a

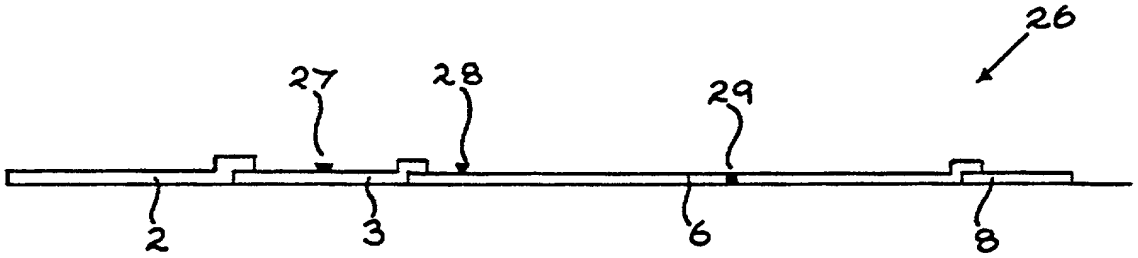


Fig 9b

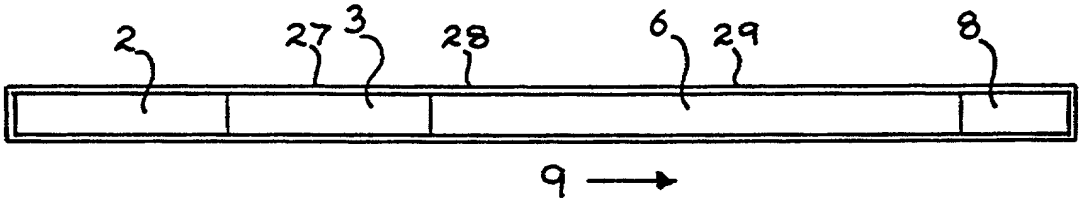


Fig 10a

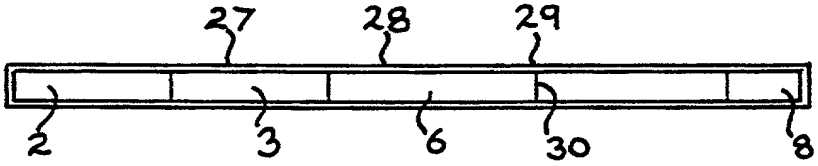
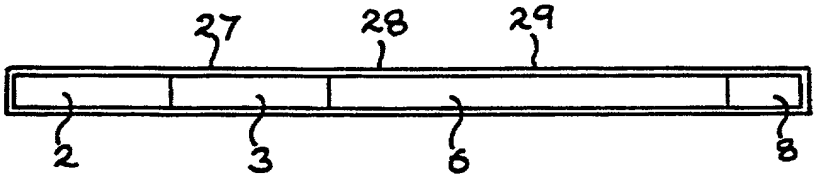


Fig 10b



DECLARATION AND POWER OF ATTORNEY

FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Assays for Autoantibodies

the specification of which was filed on 27th October 1999 as PCT patent application GB99/03548.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations. §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign applications for patent listed below, and I have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

United Kingdom	9823397.6	27th October 1998

And I hereby appoint:

Kenneth I. Kohn of Kohn & Associates, 30500 Northwestern Hwy., Suite 410, Farmington Hills, Michigan 48334, as my attorney, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark office connected therewith. I hereby revoke any and all previous declarations and powers of attorney signed in connection with the subject application

Please address all communications, and direct all telephone calls regarding this application, to:

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Kohn & Associates
30500 Northwestern Hwy
Suite 410
Farmington Hills
MI 48334

Tel: (248) 539-5050

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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Dated this 22nd day of May 2000

P. J. Burne.

Peter John Burne

2-00

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Dated this 26th day of May 2000

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